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Salvage of blood urea nitrogen in sheep is highly dependent on plasma urea concentration and the efficiency of capture within the digestive tract^{1,2}

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ABSTRACT: The aims of this study were 1) to determine whether transfer of blood urea to the gastrointestinal tract (GIT) or the efficiency of capture of urea N within the GIT is more limiting for urea N salvage, and 2) to establish the relationship between plasma urea concentration and recycling of urea N to the GIT. We used an i.v. urea infusion model in sheep to elevate the urea entry rate and plasma concentrations, thus avoiding direct manipulation of the rumen environment that otherwise occurs when feeding additional N. Four growing sheep (28.1 ± 0.6 kg of BW) were fed a low-protein (6.8% CP, DM basis) diet and assigned to 4 rates of i.v. urea infusion (0, 3.8, 7.5, or 11.3 g of urea N/d; 10-d periods) in a balanced 4×4 Latin square design. Nitrogen retention (d 6 to 9), urea kinetics ($[^{15}\text{N}_2]$ urea infusion over 80 h), and plasma AA were determined. Urea infusion increased apparent total

tract digestibility of N (29.9 to 41.3%) and DM (47.5 to 58.9%), and N retention (1.45 to 5.46 g/d). The plasma urea N entry rate increased (5.1 to 21.8 g/d) with urea infusion, as did the amount of urea N entering the GIT (4.1 to 13.2 g/d). Urea N transfer to the GIT increased with plasma urea concentration, but the increases were smaller at greater concentrations of plasma urea. Anabolic use of urea N within the GIT also increased with urea infusion (1.43 to 2.98 g/d; $P = 0.003$), but anabolic use as a proportion of GIT entry was low and decreased (35 to 22%; $P = 0.003$) with urea infusions. Consequently, much (44 to 67%) of the urea N transferred to the GIT returned to the liver for resynthesis of urea (1.8 to 9.2 g/d; $P < 0.05$). The present results suggest that transfer of blood urea to the GIT is 1) highly related to blood urea concentration, and 2) less limiting for N retention than is the efficiency of capture of recycled urea N by microbes within the GIT.

Key words: digestive tract, nitrogen metabolism, ruminant, urea kinetics

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INTRODUCTION

In ruminants, many common feeding conditions lead to a hepatic urea N production rate equaling, and often exceeding, apparent digestible N intake (Sarraseca et al., 1998; Lobley et al., 2000; Lapierre and Lobley, 2001). Even in these situations, the animals were able to achieve a positive N balance, in part by maintaining high rates of urea N transfer to and utilization within the gastrointestinal tract (GIT), particularly within the rumen. It is therefore clear that significant advances

in improving the efficiency of N utilization of ruminants will rely on an ability to promote urea N recycling and salvage under a range of dietary and physiological situations.

In recent years, use of $[^{15}\text{N}_2]$ urea has allowed urea N to be traced from the blood to its various metabolic fates (sheep: Sarraseca et al., 1998; Lobley et al., 2000; Marini et al., 2004; beef cattle: Archibeque et al., 2001; Archibeque et al., 2002; Marini and Van Amburgh, 2003; dairy cows: Lapierre and Lobley, 2001; Ruiz et al., 2002). From these studies, it is clear that the rate of urea N transfer to the GIT and the efficiency of its utilization by GIT microbes were key determinants of the overall efficiency of the urea N salvage process. Furthermore, although plasma urea concentration would be expected to have a major influence on urea transfer to the GIT, correlations between the two are often low (Harmeyer and Martens, 1980; Lapierre and Lobley, 2001); therefore, the roles of urea transport

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systems should be considered (Marini and Van Amburgh, 2003; Marini et al., 2004; Stewart et al., 2005).

The objectives of this study were 1) to determine whether transfer of blood urea to the GIT or the efficiency of capture of urea N within the GIT is more limiting for salvage of urea N for tissue N gain, and 2) to establish the relationship between plasma urea concentration and urea N recycled to the GIT, independent of diet-induced events in the rumen.

MATERIALS AND METHODS

To address these objectives, we used an i.v. urea infusion model in growing sheep. By infusing increasing amounts of urea, we artificially elevated the urea entry rate and plasma concentrations without directly manipulating the rumen environment, which otherwise would occur if additional N were supplied by the diet. In doing so, we aimed to minimize the influences, both negative and positive, on urea N recycling resulting from events occurring within the GIT environment, thus allowing a less complicated examination of the potential of and limits to urea N recycling. Partition of urea N toward its various metabolic fates was determined using an [$^{15}\text{N}_2$]urea tracer approach and a kinetic model (Sarraf et al., 1998; Lobley et al., 2000).

Sheep, Diets, and Experimental Protocol

The experimental protocol was approved by the Animal Care and Use Committee of the University of Maryland. Four Polypay \times Dorsett wether sheep (28.1 ± 0.6 kg of BW) were randomly assigned to 1 of 4 rates of urea delivery (0.34, 8, 16, or 24 g of urea/d, equivalent to 0.16, 3.76, 7.52, and 11.28 g of N/d) via an i.v. infusion (in 350 mL of 0.15 M NaCl) in a balanced 4×4 Latin square design. Each treatment period lasted 10 d, during which the wethers were housed in individual metabolic crates. Between periods, the wethers were placed in floor pens for 4 d for exercise. Throughout the experiment, wethers were fed a pelleted diet (906 g of DM/d, $\sim 1.5\times$ the maintenance energy requirement; AFRC, 1992) that was low in protein (6.8% CP, DM basis) but contained moderate (9 MJ of ME/kg of DM) fermentable energy (Table 1). The ration was delivered in equal amounts every 2 h by automated feeders, and fresh water was provided daily.

The urea infusion rates were selected to cover a range of MP supplies and were expected to raise plasma urea concentrations. Thus, the wethers were fed a basal diet deficient in MP intake by 35% with no urea infused (control, ~ 60 g of MP/d), whereas the greatest rate of urea infusion (equivalent to ~ 70 g of MP/d) increased the potential MP supply to 140% of the predicted requirement (92 g of MP/d; AFRC, 1992) for sheep gaining 250 g of BW/d. A unit of infused urea N was assumed to be equivalent to a unit of MP N if 100% of the infused urea was transferred to the GIT for microbial protein synthesis.

Table 1. Composition of the experimental diet

Item	Amount, g/kg of diet (DM basis)
Ingredient	
Corn, dent yellow grain	285
Cottonseed hulls	135
Beet pulp, dried	200
Wheat straw	365
Vitamin-mineral premix ¹	15
Nutrient composition (by chemical analysis)	
DM	906
CP	76
ADF	367
NDF	516
Starch	232
Crude fat	24
TDN	680
NE _m , MJ/kg	6.13
NE _g , MJ/kg	3.66

¹Shepherd's Pride (Renaissance Nutrition, Inc., Roaring Spring, PA), which provided (per kg of premix): Ca, 220 g; NaCl, 160 g; S, 31 g; P, 30 g; Mg, 27 g; K, 24 g; Fe, 1,820 mg; Zn, 2,700 mg; Mn, 240 mg; I, 40 mg; Co, 35 mg; Se, 24 mg; vitamin A, 682,799 IU; vitamin D, 137,574 IU; and vitamin E, 1,774 IU.

Wethers were fitted with a temporary jugular vein catheter 2 d before each treatment period (El-Kadi et al., 2006). The infusion solutions of unlabeled urea were filtered through a 0.45- μm filter (Nalgene, Rochester, NY) into a sterile glass bottle and infused i.v. through a sterile inline syringe filter (0.2 μm , Nalgene, Rochester, NY). [$^{15}\text{N}_2$]Urea (99 atom percent ^{15}N , Cambridge Isotope Laboratories, Inc., Andover, MA) was infused (last 80 h of the 10-d period) in place of an equal amount of the unlabeled urea, ensuring that the plasma urea pool size remained stable throughout the 10 d of urea infusion. The amount of [$^{15}\text{N}_2$]urea included in the infusate for each treatment (0.34, 0.53, 0.79, or 1.02 g/d) was predicted to increase the urinary [$^{15}\text{N}_2$]urea enrichment to ~ 3 atom percent excess (APE) at plateau (steady state). During the control period, unlabeled urea was also infused (0.34 g/d) during the period preceding the [$^{15}\text{N}_2$]urea infusion to account for the equivalent amount of [$^{15}\text{N}_2$]urea infused during the last 80 h. During the last 5 d of each treatment period, wethers were fitted with a harness and bag for total collection of feces, and urine was collected under a slight vacuum into a sealed vessel containing 100 mL of 4 M HCl.

Sample Collection and Analysis

Total urine and feces were collected daily, mixed thoroughly, and subsamples (5% of urine and 20% of feces) were stored at -20°C until analyzed. Over the last 10 h of isotope infusion, all feces were collected and urine was collected over 2-h intervals. Urea concentration was determined by isotope dilution with gas chromatography-mass spectrometry (El-Kadi et al., 2006). To a known weight (100 mg) of urine was added an equivalent weight of a solution of [^{13}C , $^{15}\text{N}_2$]urea (5 mg/g,

Cambridge Isotope Laboratories, Inc.). For urine [^{15}N]urea enrichment, a separate aliquot of urine was processed.

Urinary urea was isolated by cation-exchange (AG 50W-X8 resin, 100 to 200 mesh, Bio-Rad Laboratories, Hercules, CA) chromatography and eluted from the resin with 4 vol of 2 M NH_4OH followed by 2 vol of water. A portion (20 to 100 μL) of the eluate was dried under N_2 gas, and urea was converted to the tertiary-butyldimethylsilyl derivative before gas chromatography-mass spectrometry (5973N mass selective detector coupled to a 6890 Series gas chromatography system, Agilent, Palo Alto, CA) under electron ionization conditions (El-Kadi et al., 2006). Selective ion monitoring was performed for ions at m/z (mass-to-charge) of 231, 232, 233, and 234, corresponding to unlabeled ($^{14}\text{N}^{14}\text{N}$), single- ($^{14}\text{N}^{15}\text{N}$), double- ($^{15}\text{N}^{15}\text{N}$), and triple-labeled (^{13}C , $^{15}\text{N}_2$, internal standard) urea. Urea concentrations were corrected for the proportions of $^{14}\text{N}^{15}\text{N}$ and $^{15}\text{N}^{15}\text{N}$ urea, and the spillover effects of m/z 233 ($^{15}\text{N}^{15}\text{N}$ urea) to 234 (^{13}C , $^{15}\text{N}_2$, internal standard) were accounted for in the calculations (Wolfe, 1992). Enrichment data are presented as APE.

Fecal samples were freeze-dried, pulverized in a liquid-nitrogen freezer mill (Freezer-Mill 6850, Spex CertiPrep Inc., Metuchen, NJ), and analyzed for total ^{15}N enrichment using a continuous flow-isotope ratio mass spectrometer (Mulvaney and Liu, 1991). Total N content in urine and feces was determined using an automated N analyzer (CN-2000, Leco, St. Joseph, MI).

Blood samples (4 mL) were collected at 1-h intervals over the last 8 h of isotope infusion. Plasma was harvested by centrifugation ($2,000 \times g$ for 15 min; 4°C) and an aliquot (0.5 g) was stored at -20°C for determination of AA and urea. To a known weight (0.5 g) of plasma was added an equivalent weight of a solution containing 0.75 mg of hydrolyzed [^{13}C]algae protein powder (99 atom percent ^{13}C , Martek Biosciences Corp., Columbia, MD), 100 nmol [indole- D_5]tryptophan, 25 nmol [methyl- D_3]methionine, and 7 μmol [^{13}C , $^{15}\text{N}_2$]urea, and the samples were stored at -20°C . Subsequently, these samples were thawed and processed for measurement of AA and urea concentrations by isotope dilution with gas chromatography-mass spectrometry (El-Kadi et al., 2006).

Kinetic Calculations

Urea N kinetics was calculated using the model described by Loble et al. (2000). This model requires measurement of [$^{14}\text{N}^{14}\text{N}$], [$^{14}\text{N}^{15}\text{N}$], and [$^{15}\text{N}^{15}\text{N}$]urea enrichment and excretion in urine, and total ^{15}N excretion in feces. Urea N entry rate (**UER**) is the sum of hepatic urea synthesis plus the urea infused as treatments. Gut N entry rate (**GER**; recycling) is the difference between UER and urinary urea N excretion (**UUE**). The partition of GER between return to the hepatic ornithine-urea cycle (**ROC**), excretion of N originating from urea into feces (**UFE**), and urea N utilized

for anabolism (**UUA**) was calculated according to the model. This model also accommodates multiple (infinite) entries of [$^{14}\text{N}^{15}\text{N}$]urea into the GIT, which, if not corrected, leads to an underestimation of ROC and an overestimation of UUA.

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC), with urea infusion treatment as the fixed effect, and wethers and period as the random effects. The results are presented as least squares means \pm SEM for each treatment. When a significant treatment effect was detected, means were compared using the Tukey-Kramer multiple comparison test. Differences were considered significant at $P < 0.05$.

RESULTS

Wethers

No feed refusals were recorded, and all wethers gained weight (range: 2.5 to 5.5 kg) during the 2 mo of the study. Urea flux data were omitted for 1 wether receiving the 16 g of urea infusion because of a power failure during tracer infusion.

Digestion and Nitrogen Balance

Apparent total tract digestibility of DM (48 to 59%; $P = 0.002$) and N (30 to 41%; $P = 0.016$) increased with urea infusion (Table 2). For the control treatment, wethers were in positive N balance (1.45 g of N retained/d), and N retention increased ($P < 0.001$) to a maximum (5.46 g of N retained/d) at the greatest level of urea infusion (11.28 g of urea N/d). Therefore, at the greatest level of urea infused, the wethers retained ~36% of the additional N infused.

Urea Nitrogen Kinetics

For all treatments, urinary [$^{15}\text{N}^{15}\text{N}$]urea (**[M+2]**) attained an isotopic plateau between 8 to 32 h (sample interval) of [$^{15}\text{N}^{15}\text{N}$]urea infusion (Figure 1A). In contrast, urinary [$^{14}\text{N}^{15}\text{N}$]urea (**[M+1]**) enrichment (not shown) continued to increase until reaching a plateau over the last 72 to 80 h of tracer infusion. Thus, for all treatments, the ratio of urinary [$^{14}\text{N}^{15}\text{N}$]:[$^{15}\text{N}^{15}\text{N}$]urea (**[M+1]:[M+2]**) was at plateau over the 72 to 80 h sampling period (Figure 1B), and these values were used in calculations. Fecal total ^{15}N enrichments did not attain a definite plateau after 80 h of tracer infusion (Figure 2), and with 4 sampling points it was not possible to predict plateau values. Therefore, the final fecal ^{15}N enrichment values over the 72 to 80 h of sampling and the average fecal N excretion over the last 4 d of treatment were used in calculations.

As expected, both UER and plasma urea concentration increased ($P < 0.001$) with increased rate of urea

Table 2. Nitrogen balance in growing wethers (n = 4) fed a low-protein diet and infused with 4 rates of urea into the jugular vein¹

Item	Urea infusion rate, g/d				SEM	P-value
	0	8	16	24		
Balance measurements, g/d						
N intake	10.67	10.67	10.67	10.67	—	—
Infused urea N ²	0.16	3.76	7.52	11.28	—	—
N in feces	7.48 ^a	6.61 ^{ab}	6.38 ^b	6.26 ^b	0.370	0.015
N in urine	2.21 ^d	4.12 ^c	6.63 ^b	10.23 ^a	0.327	<0.001
Urine urea N	0.94 ^d	2.48 ^c	4.95 ^b	7.79 ^a	0.256	<0.001
Urea N of total urine N, %	40.4 ^c	59.6 ^b	73.2 ^a	80.2 ^a	3.43	<0.001
N retention	1.14 ^c	3.69 ^b	5.18 ^{ab}	5.46 ^a	0.446	<0.001
Total tract N digestibility, %	29.9 ^b	38.0 ^{ab}	40.2 ^a	41.3 ^a	3.47	0.015
Total tract DM digestibility, %	47.5 ^b	55.0 ^a	56.0 ^a	58.9 ^a	2.73	0.002

^{a-d}Within a row, means without a common superscript letter differ (Tukey-Kramer test, $P < 0.05$).

¹Nitrogen balance components were measured during the last 4 d of each 10-d treatment period. Values are least squares means.

²During the control period, 0.16 g/d of unlabeled urea N was infused to account for the equivalent amount of [¹⁵N₂]urea infused over the last 80 h of the 10-d treatment period, during which the N kinetics measurements were made.

infusion (Table 3). The quantity of urea N partitioned to the GIT also increased (GER; $P < 0.001$) as did the UUE ($P < 0.001$). The GER and plasma urea concentration were positively correlated, and this relationship was best described by a second-order polynomial equation (Figure 3). The GER as a proportion of UER declined ($P = 0.014$) from 0.813 for the control to 0.628 for the 24 g/d urea infusion. Reciprocally, UUE accounted for an increasing proportion of UER ($P = 0.014$) with urea infusion level. Despite this, wethers partitioned ~86% of the infused urea N to the GIT at the greatest rate of urea infusion. Of the urea N transferred to the GIT, much was returned to the ROC for resynthesis of urea, and this increased ($P < 0.001$) on an absolute and fractional basis with each level of urea infused. The efficiency of utilization of GER for anabolic purposes (GER to UUA) steadily declined ($P = 0.003$) with urea infusion, and on an absolute basis, UUA was maximal when 8 g/d of urea was infused. Urea N transferred to feces represented the smallest proportion of GER (11 to 21%). Although UFE increased ($P < 0.005$) with increasing urea infusion, the value at the greatest urea infusion rate was greater by only 0.66 g of N/d compared with the control.

Plasma AA Concentrations

There was a general trend for plasma concentrations of essential AA to increase with increasing urea infusion (Figure 4). In particular, concentrations of leucine ($P = 0.02$), lysine ($P = 0.050$), methionine ($P = 0.050$), phenylalanine ($P = 0.008$), and tryptophan ($P = 0.016$) were elevated, reaching a maximum concentration when 16 g/d of urea was infused. These trends for essential and potentially limiting AA were also consistent with the observed increases in N retention. For the nonessential AA, urea infusion increased the concentration of proline ($P = 0.047$).

DISCUSSION

In the current study, all wethers were in positive N balance during the control period (1.45 g of N/d, $P < 0.05$), in which only the basal diet supplied available N. Positive N balance was achieved despite the fact that UER (5.05 g of N/d) far exceeded apparent digestible N intake (3.19 g of N/d). Under these conditions, the wethers recycled 81% of plasma urea entry to the GIT, where 35% of GER was utilized for anabolic purposes, such as for microbial protein synthesis and AA absorption. It is not uncommon to find situations in growing ruminants in which hepatic ureagenesis exceeded apparent digestible N intake (sheep: 1.20 to 1.47:1; Sarra-seca et al., 1998; Lobley et al., 2000; steers: 1.07:1; Archibeque et al., 2001), yet the animals were able to meet the requirements for protein to support both maintenance and growth. At the extreme, wild ruminants such as elk (*Cervus elaphus nelsoni*) are able to survive on low-quality forages despite even greater disparities between hepatic ureagenesis and digestible N intake (e.g., 2.83 to 4.87:1; Mould and Robbins, 1981).

The quantity of urea N transferred to the GIT increased with each increase in the rate of urea infusion (Table 3). Regarding what “drives” urea transfer to the GIT, for some time it had been assumed that urea transfer across the rumen wall occurs by simple diffusion mechanisms from blood (Haupt and Haupt, 1968), and indeed, greater plasma urea concentrations are associated with greater transfer across and urea N recycling to the rumen (Harmeyer and Martens, 1980). However, in their evaluation of a large database of studies conducted in sheep and cattle, Lapierre and Lobley (2001) found plasma urea concentrations to have low correlations ($r^2 < 0.20$) with the amount of urea N recycled to the GIT. Those correlations were based on studies in which total N or feed intake was manipulated; therefore, other counteractive mechanisms may have been

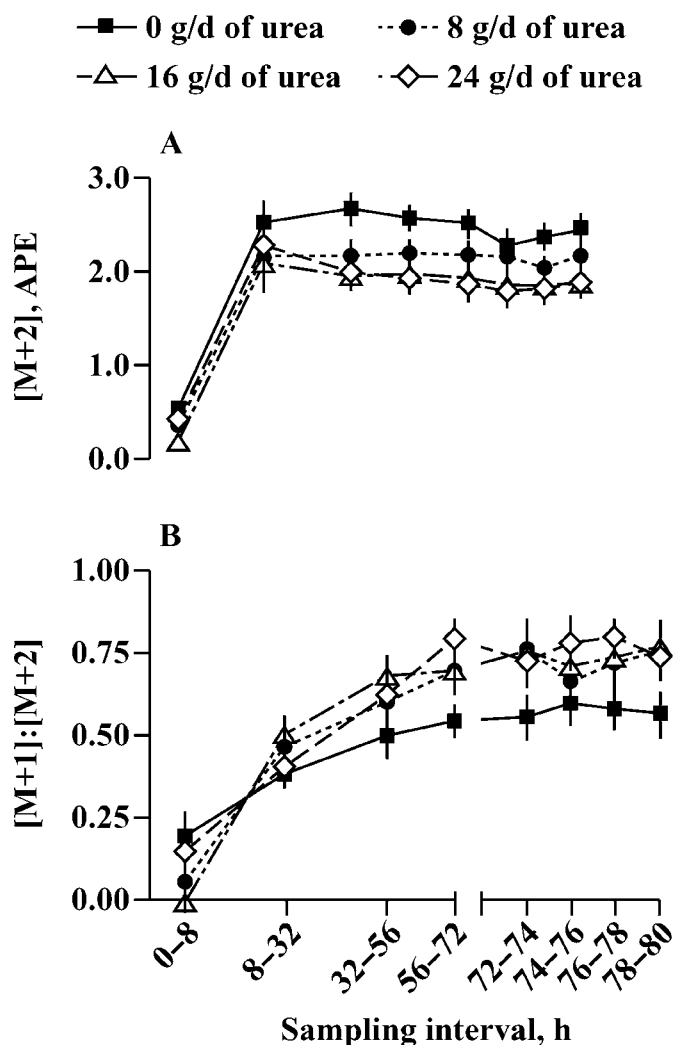


Figure 1. The time-course of (A) enrichment of urinary $[^{15}\text{N}^{15}\text{N}]$ urea (M+2) and (B) the ratio of $[^{14}\text{N}^{15}\text{N}]:[^{15}\text{N}^{15}\text{N}]$ urea (M+1:M+2) in urine collected during the 80-h infusion of $[^{15}\text{N}^{15}\text{N}]$ urea. Each line represents a treatment (urea infusion rate, g/d), and the symbols represent the mean of 4 wethers (bars = SEM). APE = atom percent excess.

operating that limited urea transfer at the blood-tissue interface. For example, elevating the rumen fluid concentrations of CO_2 , butyrate, and ammonia each have been shown to reduce urea transfer across the rumen wall (Rémond et al., 1993b), and all of these are directly influenced by the type and level of feed intake (Rémond et al., 1993a). Furthermore, with the recent discovery that a facilitative urea transporter is expressed in bovine (Marini and Van Amburgh, 2003; Stewart et al., 2005) and ovine (Marini et al., 2004) GIT tissues, and that this mechanism accounts for much of the urea transported across rumen tissues (Stewart et al., 2005), regulation of urea transfer to the GIT by the host animal is a possibility that now requires consideration.

In the current study, we purposely enhanced plasma urea concentration by i.v. infusion to avoid direct ma-

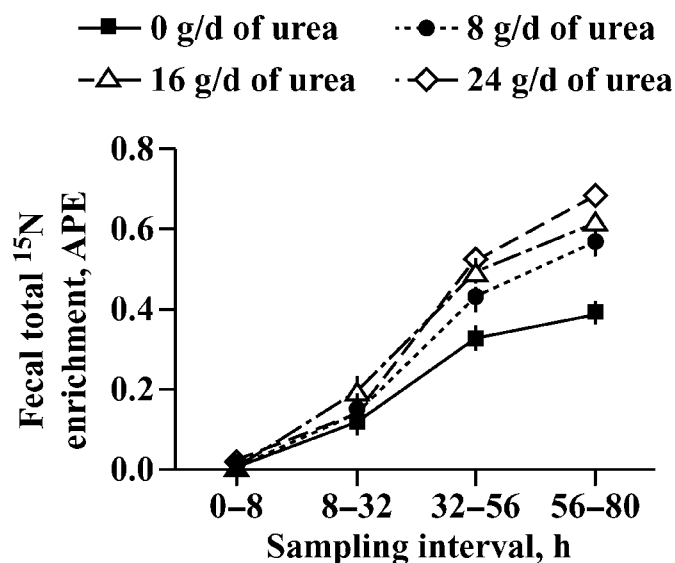


Figure 2. The time-course of total ^{15}N enrichment in composite fecal samples during the 80-h infusion of $[^{15}\text{N}^{15}\text{N}]$ urea. Each line represents a treatment (urea infusion rate, g/d), and the symbols represent the mean of 4 wethers (bars = SEM). APE = atom percent excess.

nipulation of the rumen environment, thus minimizing the potential inhibitory actions of fermentation end-products on plasma urea transfer to the GIT, be they by transporter or diffusion mechanisms. Contrary to previous evaluations, we observed a high correlation ($r^2 = 0.95$) between plasma urea concentration and GER that was best described by a second-order polynomial equation (Figure 3). Based on this analysis, our data indicate that within the physiological range of plasma urea (2 to 5 mM), the mechanisms (diffusion, transporter mediated) of urea transfer to the GIT are probably operating at a high capacity and impose minimal constraints on urea transfer to the GIT. At the greater concentrations of plasma urea, however, it was apparent that transfer mechanisms became more limiting, but not to the extent that the quantity of urea N transfer was reduced. This may have involved urea transporters or limitations on diffusion created by "boundary layer effects" from the local buildup of rumen ammonia (Egan et al., 1986). In fact, because rumen ammonia concentration is highly correlated with plasma urea concentration (Kennedy and Milligan, 1978), this mechanism of inhibition may have become more influential at the greater concentrations of plasma urea observed herein. Involvement of urea transporter mechanisms in promoting or limiting urea recycling to the GIT of ruminants remains equivocal. For example, urea transporter B mRNA expression was found to be greater in cattle fed at high protein intakes (Marini and Van Amburgh, 2003), whereas no change in the protein abundance of this transporter was observed in sheep fed increasing levels of dietary protein (Marini et al., 2004). Furthermore, a carrier-mediated facilitative

Table 3. Urea N fluxes in growing wethers (n = 4) fed a low-protein diet and infused with 4 rates of urea into the jugular vein

Item ¹	Urea infusion rate, ² g/d				SEM	P-value
	0	8	16	24		
Plasma urea, mM	1.27 ^d	2.54 ^c	4.28 ^b	5.93 ^a	0.232	<0.001
Urea N flux, g/d						
UER	5.05 ^d	10.56 ^c	16.98 ^b	21.78 ^a	0.619	<0.001
UUE	0.95 ^d	2.54 ^c	5.44 ^b	8.11 ^a	0.304	<0.001
GER	4.11 ^d	8.02 ^c	11.28 ^b	13.67 ^a	0.507	<0.001
ROC	1.82 ^d	4.42 ^c	7.40 ^b	9.18 ^a	0.405	<0.001
UFE	0.86 ^c	1.18 ^b	1.48 ^{ab}	1.52 ^a	0.102	0.002
UUA	1.43 ^b	2.42 ^a	2.50 ^a	2.98 ^a	0.320	0.003
Fractional transfer of urea N						
UER to urine	0.187 ^c	0.244 ^{bc}	0.327 ^{ab}	0.372 ^a	0.023	0.014
UER to GIT	0.813 ^a	0.756 ^{ab}	0.673 ^{bc}	0.628 ^c	0.023	0.014
GER to ROC	0.439 ^c	0.550 ^b	0.657 ^{ab}	0.672 ^a	0.029	<0.001
GER to UFE	0.211 ^a	0.152 ^b	0.131 ^{bc}	0.111 ^c	0.016	<0.001
GER to UUA	0.350 ^a	0.298 ^{ab}	0.218 ^{bc}	0.218 ^c	0.024	0.003
Urinary urea clearance, ³ mL/(min·kg of BW)	0.68 ^c	0.88 ^b	1.16 ^a	1.18 ^a	0.08	0.005

^{a-d}Within a row, means without a common superscript letter differ (Tukey-Kramer test, $P < 0.05$).

¹UER = plasma urea entry rate; UUE = urinary urea elimination; GER = gut entry rate; ROC = return to ornithine cycle; UFE = urea N transferred to feces; UUA = urea N utilized for anabolism; GIT = gastrointestinal tract.

²Equivalent rates of N infusion were: 0 g of urea, 0.16 g of N; 8 g of urea, 3.76 g of N; 16 g of urea, 7.52 g of N; 24 g of urea, 11.28 g of N.

³Calculated as: (urinary urea output/plasma urea concentration)/BW.

urea transport mechanism identified in the rumen epithelia of sheep was found to act as a bidirectional transport mechanism (Ritzhaupt et al., 1997; Ritzhaupt et al., 1998), so the relationship of this transport mechanism to the transfer or efflux of urea by the rumen tissues is still in question. To date, the kidneys, liver, colon, small intestines, and red blood cells have been found to express several forms of urea transporters, all

of which are selective, facilitative (non-energy-dependent) transporters and none of which are saturated at 200 mM urea (Smith and Rousselet, 2001). Given the latter observation, the present results suggest that urea transfer to the GIT of ruminants may not be limited or saturated across the physiological range of plasma urea concentrations (2 to 5 mM) as were observed in the current study. Notwithstanding, under normal feeding conditions in which dietary energy or protein is manipulated, the consequent changes in rumen fermentation and GIT microbial ecology will undoubtedly have an influence on urea transport across and use within the GIT (e.g., Marini and Van Amburgh, 2003). However, in the current study, the capacity to recycle urea N to the GIT (GER) was high and continued to increase with each level of urea infused.

Concomitant with the increase in GER when plasma urea concentration was elevated, we also observed an increase in the utilization of recycled urea N by rumen microbes, as evidenced by the elevation in plasma concentrations of limiting AA (lysine and methionine; Storm and Ørskov, 1984) and, in a related manner, increased whole body N retention. Conventional wisdom holds that conversion of recycled urea N into microbial protein and, ultimately, AA absorption, can occur only when urea N is transferred to the rumen (Egan et al., 1986; Mosenthin et al., 1992). At the greatest rate of urea infusion, an additional 4.01 g of N was retained from the net addition of 11.12 g of urea N. Although this 36% conversion efficiency would be impressive for ruminants fed additional protein, much greater efficiencies of use for recycled urea N were possible based on our data. Thus, whereas 108, 87, and 64% of the

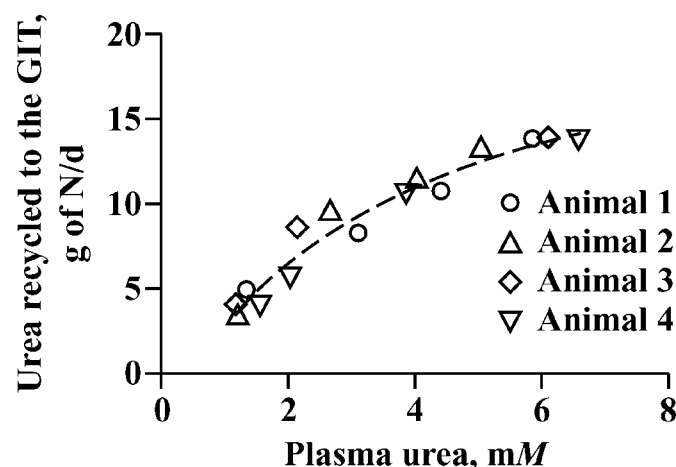


Figure 3. Plot of urea N recycled to the gastrointestinal tract (GIT; y-axis) against plasma urea concentration (x-axis) in growing wethers (n = 4) fed a low-protein diet and infused with 4 rates of urea into the jugular vein (0.16, 3.76, 7.52, and 11.28 g of N/d) for 10 d. The dashed line represents the second-order regression equation; $y = 0.0377 + 3.66(x) - 0.2377(x^2)$, $r^2 = 0.95$, $P < 0.001$.

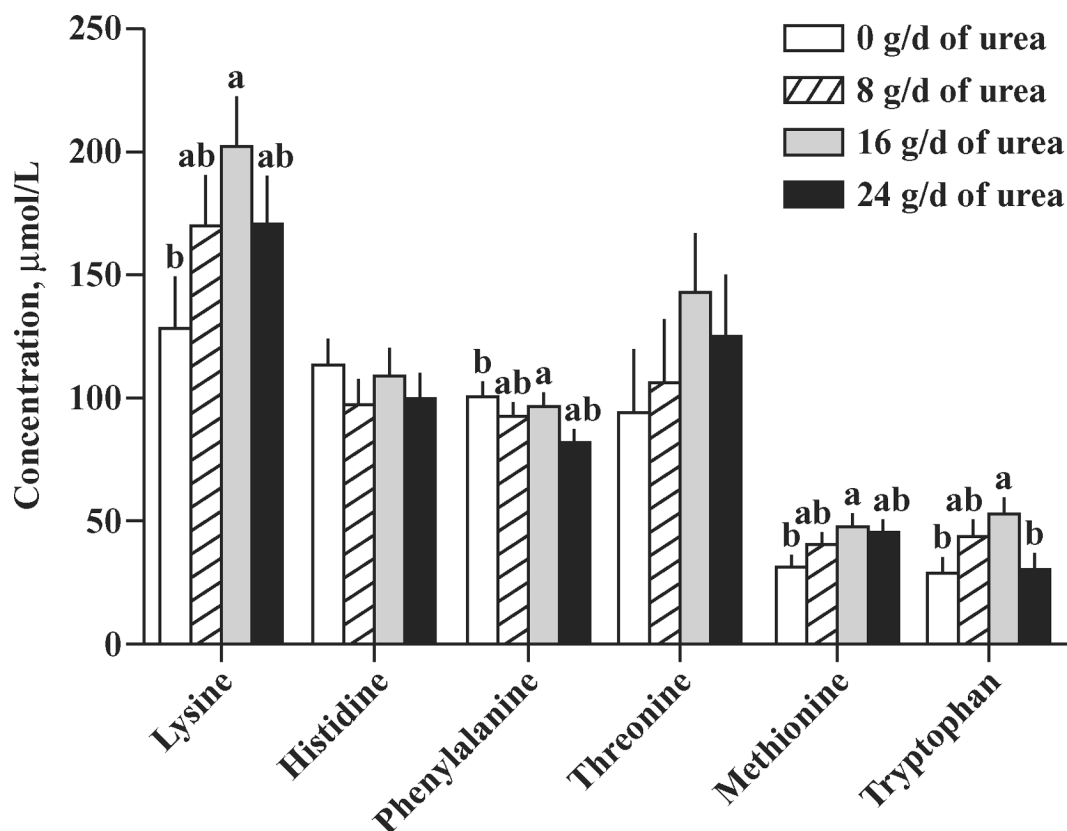


Figure 4. Plasma concentrations of some essential AA in growing wethers ($n = 4$) fed a low-protein diet and infused with 4 rates of urea into the jugular vein for 10 d. For each AA, bars without a common superscript letter differ ($P < 0.05$). Error bars = SEM.

incremental increases in urea N infused were transferred to the GIT (Table 3), this additional supply of N to the GIT was partly offset by the increasing proportion (44 to 67%) and quantity (1.8 to 9.2 g of urea N) of GER that was hydrolyzed and returned to the liver for resynthesis of urea. Concomitantly, kidney clearance of urea [$\text{mL}/(\text{min} \cdot \text{kg of BW})$; Table 3] increased with the rate of urea infusion, which probably contributed to the decreasing proportion of blood urea entry partitioned to the GIT. In consequence, the utilization efficiency of recycled urea N within the GIT declined from 35% for the control to 22% at the greatest level of urea infused. We interpreted this as demonstrating that capture and use of recycled urea N within the GIT is more rate limiting to the N salvage process than is the rate of urea N transfer to the GIT from blood or saliva. Clearly, the potential exists to realize significant improvements in N efficiency by ruminants by increasing microbial use of recycled urea N.

Fecal excretion accounted for 21% of GER in the control period, decreasing to 11% of GER at the greatest urea infusion rate. These may represent an underestimation because fecal ^{15}N enrichments did not attain a definable plateau at the conclusion of the 80-h tracer infusion. Because anabolic use (UUA) is calculated from the difference of ROC and UFE, failure to achieve plateau in fecal ^{15}N will result in a reciprocal decrease in

the estimate of urea N used for anabolic purposes. For example, assuming a further increase of 30% in fecal ^{15}N plateau values for the greatest level of urea infusion, the quantitative significance to fecal urea N transfer equates to an additional 0.45 g of UFE, and an increase in the proportion of GER to UFE from 11 to 14%. In consequence, anabolic use will be reduced from 2.98 to 2.53 g/d (Table 3); likewise, the proportion GER to UUA will be reduced from 21.8 to 19%. Failure of fecal ^{15}N to attain a definite plateau does not affect GER or the proportion of urea entry rate transferred to the GIT.

Fecal N excretion decreased with urea infusion, despite the small increase in UFE. This reduction in fecal N excretion was the result of improvements in dietary DM and N digestibility, in addition to the increases observed in anabolic use of recycled urea N and in whole body N retention. Similar improvements in DM and N digestion have been observed in response to increased dietary protein intake (Ruiz et al., 2002; Marini and Van Amburgh, 2003; Marini et al., 2004), which probably relates to correction of the N (ammonia) deficiency of the microbes in the rumen (Smith, 1979). Taken together, it seems likely that either most of the additional urea N transferred to the GIT was directed to the rumen or that the urea N was transferred to postrumen compartments where it was hydrolyzed and absorbed as

ammonia, rather than being incorporated into microbial protein in feces.

In conclusion, the results of the current study suggest that the transfer of urea N to the GIT is highly related to plasma urea concentration and that the transfer process (transporter mediated or diffusion) does not limit urea N salvage as much as the efficiency of capture of the recycled urea N within the GIT. The latter is influenced by microbial fermentation events occurring within the GIT (e.g., pH, CO₂, butyrate, ammonia vs. ammonium, soluble carbohydrates) and other processes (e.g., blood vs. saliva transfer, rumen motility and passage rate) associated with the rumen physiology. One proviso to this conclusion, however, is that our measurements were of urea transfer to the whole GIT. Thus, urea hydrolysis probably occurred all throughout the GIT, with capture for microbial protein synthesis and AA absorption possible only from blood urea N transferred to the rumen. That being the case, the next advances that improve the efficiency of use of urea N recycled to the GIT will be based on knowledge of the GIT compartments to which urea is transferred and, more important, the composition of the microbial ecosystems that reside there.

LITERATURE CITED

- AFRC (Agricultural and Food Research Council). 1992. Technical Committee on Responses to Nutrients. Report No. 9. Nutritive Requirements of Ruminant Animals: Protein. Nutr. Abstr. Rev. B 62:803–817.
- Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2002. Nitrogen metabolism of beef steers fed endophyte-free fescue hay: Effects of rumen protected methionine supplementation. *J. Anim. Sci.* 80:1344–1351.
- Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2001. Urea flux in beef steers: Effects of forage species and nitrogen fertilization. *J. Anim. Sci.* 79:1937–1943.
- Egan, A. R., K. Boda, and J. Varady. 1986. Regulation of nitrogen metabolism and recycling. Pages 386–402 in *Control of Digestion and Metabolism in Ruminants*. L. P. Milligan, W. L. Grovum, and A. Dobson, ed. Prentice-Hall, Englewood Cliffs, NJ.
- El-Kadi, S. W., R. L. Baldwin VI, N. E. Sunny, S. L. Owens, and B. J. Bequette. 2006. Intestinal protein supply alters amino acid, but not glucose, metabolism by the sheep gastrointestinal tract. *J. Nutr.* 136:1261–1269.
- Harmeyer, J., and H. Martens. 1980. Aspects of urea metabolism in ruminants with reference to the goat. *J. Dairy Sci.* 63:1707–1728.
- Houpt, T. R., and K. A. Houpt. 1968. Transfer of urea nitrogen across the rumen wall. *Am. J. Physiol.* 214:1296–1303.
- Kennedy, P. M., and L. P. Milligan. 1978. Transfer of urea from the blood to the rumen of sheep. *Br. J. Nutr.* 40:149–154.
- Lapierre, H., and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* 84:E223–E236.
- Lobley, G. E., D. M. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive [¹⁵N]¹⁵N]urea kinetics. *Br. J. Nutr.* 84:459–468.
- Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. *J. Anim. Sci.* 81:545–552.
- Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. *J. Anim. Sci.* 82:1157–1164.
- Mosenthin, R., W. C. Sauer, and C. F. M. de Lange. 1992. Tracer studies of urea kinetics in growing pigs: I. The effect of intravenous infusion of urea on urea recycling and site of urea secretion into gastrointestinal tract. *J. Anim. Sci.* 70:3458–3466.
- Mould, E. D., and C. T. Robbins. 1981. Nitrogen metabolism in elk. *J. Wildl. Manage.* 45:323–334.
- Mulvaney, R. L., and Y. P. Liu. 1991. Refinement and evaluation of an automated mass spectrometer for nitrogen isotope analysis by the Rittenberg technique. *J. Automatic. Chem.* 13:273–280.
- Rémond, D., J. P. Chaise, E. Delval, and C. Poncet. 1993a. Net flux of metabolites across the ruminal wall of sheep fed twice a day with orchardgrass hay. *J. Anim. Sci.* 71:2529–2538.
- Rémond, D., J. P. Chaise, E. Delval, and C. Poncet. 1993b. Net transfer of urea and ammonia across the ruminal wall of sheep. *J. Anim. Sci.* 71:2785–2792.
- Ritzhaupt, A., G. Breves, B. Schröder, C. G. Winckler, and S. P. Shirazi-Beechey. 1997. Urea transport in gastrointestinal tract of ruminants: Effect of dietary nitrogen. *Biochem. Soc. Trans.* 25:490S. (Abstr.)
- Ritzhaupt, A., I. S. Wood, A. A. Jackson, B. J. Moran, and S. P. Shirazi-Beechey. 1998. Isolation of a RT-PCR fragment from human colon and sheep rumen RNA with nucleotide sequence similarity to human and rat urea transporter isoforms. *Biochem. Soc. Trans.* 26:40. (Abstr.)
- Ruiz, R., L. O. Tedeschi, J. C. Marini, D. G. Fox, A. N. Pell, G. Jarvis, and J. B. Russell. 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: Evaluation of the Cornell Net Carbohydrate and Protein System ruminal nitrogen deficiency adjustment. *J. Dairy Sci.* 85:2986–2999.
- Sarraseca, A., E. Milne, M. J. Metcalf, and G. E. Lobley. 1998. Urea recycling in sheep: Effects of intake. *Br. J. Nutr.* 79:79–88.
- Smith, R. H. 1979. Synthesis of microbial nitrogen compounds in the rumen and their subsequent digestion. *J. Anim. Sci.* 49:1604–1612.
- Smith, C. P., and G. Rousselet. 2001. Facilitative urea transporters. *J. Membr. Biol.* 183:1–14.
- Stewart, G. S., C. Graham, S. Cattell, T. P. L. Smith, N. L. Simmons, and C. P. Smith. 2005. UT-B is expressed in bovine rumen: Potential role in ruminal urea transport. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289:R605–R612.
- Storm, E., and E. R. Ørskov. 1984. The nutritive value of rumen micro-organisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. *Br. J. Nutr.* 52:613–620.
- Wolfe, R. R. 1992. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. Wiley-Liss, New York, NY.

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